STUDIES ON RHIZOSPHERE MYCOFLORA OF CERTAIN CROP PLANTS

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Certificate

This is to certify that the dissertation entitled " Studies on rhizosphere mycoflora of certain crop plants " is a bonafide work carried out under my supervision by Mr. Abdul Rasheed Ansari and can be submitted in partial fulfilment of the requirements for the award of degree of Master of Philosophy in Botany.

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(ABDUL RASHEED ANSARI)

Contents

INTRODUCTION & REVIEW OF LITERATURE

The zone of greatly intesified microbial activity around the roots of green plants, which is known as rhizosphere, was discovered by L.Hiltner (1904). Fresh attention was drawn to this phenomenon some twenty five years later by the substantial researches of R.L.Starkey (1929, 1931). During the last three decades substantially rich contributions have been made in this field. It is in the rhizosphere that the soil microbiologists and plant pathologists find a region of common interest. Some terms like 'Rhizoplane' for external surface of plant root together with closely adhering particles of soil or debris as well as 'outer rhizosphere' or closer rhizosphere to designate the sites of microbial concentration have also been in use.

Rhizosphere is a highly favourable habitat for proliferation and metobolism of microorganisms. The microbial population has been intestively investigated by the microscopic, cultural and biochemical techniques. Different plant species establish different type of subterranean flora. The variation have been attributed to the difference in the rooting habits, tissues composition and excretion products of the micro-organisms. The primary root population is determined by the habit created by the plants and the secondary flora is, however, influenced by the activities of the intial population.

Rhizosphere microflora is influenced by the age of the plant and the environmental conditions. Timonin (1940) observed that rhizosphere microflora is established within three days of seed germination. Further development of the rhizophere population depends on the growth pattern of the plant. Usually, maximum activity is recorded when the vegetative growth is at its peek. Krassilnikov et al (1933) and Obraztzova (1935) recorded an increase in the rhizosphere microflora, when the plants were matured. Beresova (1941) observed qualitative differences at different stages of the growth of plant. Rovira (1959) observed that the rhizosphere activity of wheat reached a distinct peek at the tillering stage and the stimulation of fungi was greater than that of actinomycetes and bacteria. Agnihothrudu (1953) studied the microflora of some of the important crops plants of South India and recorded a decline in fungal numbers upto the time of the flowering of the plants. However, when the plants such as French bean, Cluster bean, seasame and Sunn-hemp started flowering there was an increase in the fungal numbers. Patel and Iyer (1961) studied the rhizosphere population of cotton plant and observed an increase in mould counts during the early stages of the growth of the plantand it extended throughout the rainy season. The population decreased after the mansoon and gradually increased thereafter showing no decline even after flowering and bailing.

Bahadur & Sinha (1965) observed a gradual increase in the fungal population with the age of plant. Gujrati (1969) observed that the fungal population of the rhizosphere of lentil & Cicer arietinum increased from the seedling stage to a maximum at flowering and fruiting then decreased, and rose again at the senescent stage when the dematiaceous fungi predominated. Kamal and Singh (1969) recorded maximum fungal population in the rhizosphere of of some ornamental plants at thetime of flowering. The influence of root on fungal flora is selective to type rather than to the total number. Continuous cultivation of a single crop frequently favour the growth of Rhizopus, Chaetomium, Aspergillus, Penicillium and Fusarium, but the genera dominating the environment vary with the crop, the soil and the climate. Agnihothrudu (1957) reported that Aspergilli were much more frequent than any other group of organisms working with the rhizosphere microflora of fifteen crop plants. Panwar et al (1967) recorded a large number of fungal species from the rhizosphere of some common desert plants colonising the sand dunes. Rhizopus, Aspergilus, Penicillium, Chaetomium, Neocosmospora, Alternaria, Helminthosporium, Curvularia, and Fusarium were some of the common genera which occured practically all the year round, while certain forms like Choanephora, Cunninghamella, Sodaria, Monocillium, Myrothecium, Cladosporium, Cylindrocephalum,

Cephalosporium, Trichothecium, Sporotrichum and Phoma varied with the plant and the season.

A number of workers have reported that there is more population of the micro-organisms in rhizosphere in comparision to non-rhizosphere. Ishizwa et al (1957), working with barley, timothy and alfalfa; Ramachandra Reddy (1959) with six types of Pteridophytes; Maliszewske and Moreau (1959) with White spruce; Ivarson and Katznelson (1960) with yellow birch; Strzelczyk (1961 a) with onion, radish , and wheat; Rovatt and Katznelson (1961) with six crop plants including flax, clover, Oats and Corn; Zagallo and Bollen (1962) with tall fescue, Rangaswami and Vasantharajan (1962) with citrus, Edward, , Swrivastava and Naim (1960) with a wide variety of crops grown in India, observed a positive rhizosphere effect. Padma and Mukherji (1972) working with Rauwolfia Serpentina observed more population in non-rhizosphere in comparision to the rhizosphere.

Substances released by the plant roots have been identified and isolated. The excreted compounds include amino-acids, simple sugars and nucleic acid derivatives. The products vary from plant to plant both in quality and quantity. The first evidence of exudates from roots was provided by Knudson (1920) who observed that peas and maize grown under aseptic conditions in sucrose solution produced considerable quantities of

reducing sugars. Knudson concluded that the sucrose was absorbed by roots and converted to reducing sugars which was excreted . Lyon and Wilson (1921) found that organic nitrogen was released from maize roots growing under sterile conditions in large capacity vessels ; they concluded that this material was released by living roots rather then the sloughed off root cells which accumulated at the bottom of the vessels. Cruner (1922) reported phospatides coming out from the roots of seedlings and mature plants. The first evidence of stimulation of specific organisms by root exudates was that of O'Brien and Prentice (1930). By showing that the cysts of the potato eelworm hatched in the presence of root washings of potato but not the washings of beet, rape, lupin, mustard and Oat roots. They demostrated the biological specificity of the exudates of different plants. Katznelson et al (1954) reported the excretion of ten aminoacids and a reducing sugar from the roots of tomatoes, soybean, barley and Oats. Rovira (1956) obtained 22 amino acids excreted from pea roots. Bhuvaneswari and Subba Rao (1957) reported different organic acids and sugars from the root exudates of Sorghum vulgare and Brassica juncea . Roy and Dwivedi (1957) listed leucine, methionine, alanine, glutamic acid, aspartic acid, Cystine, phenylalanine, arginine, rhamnose, arabinose, glucose, fructose, sucrose and raffinose as the constituents of roots exudates of some leguminous crops. The root excretion have been found to have pronounced influence on the germination of fungal spores. Barton (1957) reported the germination of Oospores

of Phythium mamillatum when they were placed in soil before growing turnip seedlings while in non-rhizosphere soil they were not able to germinate. Coley-Smith and Hickman (1957) made similar observations regarding the germination in soil of sclerotia of Sclerotium cepivorum in the presence of onion. Roy & Darvedi (1967) found complete inhibition of conidial germination of Helminthosporium sativum and Fusarium culmorum on glass slides in unsterilised soil but majority of the conidia were able to germinate when wheat seeldings were grown on the slides.

The environmental factors like moisture, temperature may exert direct or indirect effect on the rhizosphere population. Seasonal effect as reported by various wokers (Katznelson) 1946; Miller & Boolaroyd , 1962) may be considered to reflect the combined influence of all these environmental conditions. Rouatt and Katznelson (1960) reported changes in the rhizosphere of wheat with reduction of light intensity. E.A.Peterson (1961) observed that shading of plant had no significant effect on fungi colonizing roots of wheat and soybean seedlings. A detailed study on temperature effect has been carried by Rouatt, Peterson et al (1963) with wheat and soybean grown at three ranges of temperatures. The results suggest that temperature exert both direct and indirect effects on the rhizosphere population, the latter being the more important. Clark (1940) and Timonin (1940) reported increased microbial counts in the rhizosphere of wheat and flax, respetively , as the soil moisture content decreased.

Clark (1948) made similar observations with soybean. Venkatesan (1962) reported highest counts of bacteria, fungi , and actinomycetes in the rhizosphere of rice in soil with 20 & 40% moisture but the R:S ratio increased with greater moisture contents until saturation ; Sondhi (1964) observed greater fungal species and population in rhizosphere mycoflora of Cicer - arietinum Linn at 15% moisture level than at 25 or 35%.

Many studies have been made on the rhizosphere flora of different soil types. E.A Peterson (1958) studied the effect of three different soil types on the rhizosphere flora of red clover roots and wheat. For red clover the R:S ratio did not vary in the three soils on the other hand for wheat a value of 34 was reported in acid sandyloam and of 10, in the neutral clay loam. Parkinson and Clark (1961) reported dominance of Fusarium over Cyclindrocarpon species on leek-roots in acid soil. Gliocladium and Mortierella species were more numerous on roots from acid soil.

There are controversial reports regarding the effect of soil treatment on the root population. A survey of the literature (Katznelson, Lochhead, and Timonin, 1948 ; Lochhead, 1959) reveals that the effect of soil treatment on the root population are often not very striking and unpredicatable. Voroshileva (1956) observed that complete mineral fertiliser had little effect on the rhizosphere population, on the other hand Mosolov, Rempe and Alexandrovskaya (1959) recorded increase counts in the root zone of winter wheat. Davey and Papavizas (1960) obtained positive

effect when the beans were planted 25 days after organic and nitrogen amendments. Samtsevich and Horisoval (1961) obtained positive results of mineral and organic fertilizers amendments on the wheat rhizosphere. Venkatesan (1962) recorded that the additions of manures (green leaf, farm yard) lowered the rhizosphere effect of rice for bacteria and fungi for the first 45 days, after which the R:S ratio increased from 4 to 21, however, the effect was very little with actinomycetes. Mishra (1972) recorded that super phosphate stimulated the rhizosphere mycoflora of Oryza sativa Linn. Jordan et al (1972) observed that strawberry rhizosphere in chitin amended soil contained an increased number of bacteria and actinomycetes. Parkash et al (1976) studied the rhizosphere mycoflora of egg plant and observed higher frequency values and fungal population in rhizosphere soil amended with urea. Low fungal population and frequency values were observed in rhizosphere soil amended with different weeds.

Some of the workers have envisaged the possibilities of improving the crop yield and plant growth by altering the rhizosphere mycoflora. Rhyzosphere mycoflora has been altered by giving certain treatments to the plant. Hellack and Cochrane (1956) recorded changes in the rhizosphere microflora of bean plants which were sprayed with bordeaux mixture. Ramachandra Reddy (1959) observed that the urea spray modified the rhizosphere of rice. Roy and Dwivedi (1967) reported that foliar spray of certain harmones

like indole acetic acid or 3-yl-propionic acid resulted increase of rhizosphere population . Singh (1971) observed changes in the rhizosphere mycoflora of soybean as a result of foliar spray of gibberellicacid, maleic hydrazide and urea. Vaidehi (1973) recorded an increase in the micro-organisms in the rhizosphere of rice as a result of foliar application of urea.

Studies on the rhizosphere effect of certain varieties of crop plants regarded by plant breeders as resistant and susceptible respectively to soil born diseases in which definite pathogens are involved has been carried out by many workers (Timonin ,1940a, b; Harper, 1950a , b; Rembouts,1933). In all cases it has been always found that the susceptible variety always had a greater rhizosphere population than the resistant. Srivastava and Mishra (1971) studied the rhizosphere mycoflora of resistant and susceptivle varieties of wheat to Puccinia graminis and observed more population of fungi in susceptible varieties. Babushkina (1973) observed more fungi in the rhizosphere of Cotton infected by Verticillium dahliae in comparison of healthy plants. Buxton (1957) working on the effect of root exudates of different pea cultivars resistant to Fusarium Oxysporum .f.pisi (Linf). Snyder and Hansen, also reported that the root exudates of the resistant cultivar affected the growth of the pathogen but he could observe no differential effect on the rate at which germ tube or mycelium of the individual races grew. However, it has been reported that the inhibition by the exudate of the resistant variety goes beyond the spore stage. Thus the rhizosphere plays the dominant role in checking the growth and survival of the pathogen and constitutes the plant's outer most defence barrier

against invasion by inhibiting the growthof the pathogen in the infection court which is a prerequisite for pathogenesis, and also by checking the multiplication of the pathogen in the rhizosphere region before a successful parasitic association is established.

Harris (1953) observed that nodulation and nitrogen fixation by a few partially effective Rhizobium strains are enhanced by simultaneous inoculation with several rhizosphere bacteria and fungi. Nilsen (1957) suggested that organic phosphorus mineralisation using glycerophosphates and nucleic acids as substrates was more rapid in rhizosphere than in soil away from the root effect. There are also evidences to Show the capacity of rhizosphere micro-organisms to alter the solubility and subsequent utilisation of manganese and iron.

Various arguments have been advanced contending that the root microflora exerts a benificial effect on plant growth, increased amount of microbial CO_2 in the root zone ; greater number , activity, and turn over of micro-organisms therein; increased solubilisation of mineral nutrients; greater synthesis of Vitamines, aminoacids , auxins, and gibberellins, which may stimulate the plant, and of antibiotics, which will protect it.

Despite the fact that Barley and Sorghum are economically important plants, yet little work has been carried out on the rhizosphere mycoflora of these crops. Hence attemps will be made to study the followings -

1. Effect of age of plant on rhizosphere and rhizoplane mycoflora of Barley and Sorghum.

2. Studies on R:S ratio at different intervals.

3. Rhizosphere and rhizoplane mycoflora of different varieties of Barley and Sorghum.

4. Rhizophere and rhizoplane mycoflora of Barley and Sorghum from 6 different soil types.

5. Rhizosphere and rhizoplane mycoflora of Barley and Sorghum in relation to foliar spray.

6. Effect of root exudates of Barley and Sorghum on certain rhizosphere fungi.

7. Comparison of different fungi in the rhizosphere and rhizoplane of Barley and Sorghum of some healthy and diseased plants.

8. Rhizosphere and rhizoplane mycoflora of Sorghum and Barley in relation to different soil amendments.

MATERIAL & METHODS

All the experiments will be performed under field conditions.

Preparation of fields:

First of all the fields will be throughly ploughed and then divided into 10 Sq.M. beds , each with separate water channels, leaving 0.5 m buffer zone between them. For each treatment there will be three beds.

Rhizosphere mycoflora of Sorghum & Barley in relations to Age and N:S ratio :-

Fungi will be isolated from the rhizosphere of these crops at intervals of 15 days from seedling stage to the maturation of the crop. Non-rhizosphere fungi will be isolated from uncultivated portion of the beds. For rhizosphere fungi analysis five plants will be carefully removed and brought to the laboratory in sterile containers. The plants will be shaken to remove superfluous soil from the root system. Under aseptic conditions each plant will be taken from its container and will be placed in a sterile glass plate. The root system will be spread out and the soil particles sticking to the root surface will be removed. The amount of soil thus obtained will be placed in sterilized petridishes. The soil will be transferred with a sterilized flattered tip of a needle into petridishes containing 10 ml. of sterilized melted and cooled peptone dextrose agar medium* (Parkinson, 1957)

* Agar	20 gm.
KH_2PO_4	1.0 gm
$Mg\ SO_4 \cdot 7H_2O$	0.5 gm
Peptone.....	5.0 gm
Dextrose.....	10.0 gm
Distilled H_2O	1000.0 ml
Rose Bengal.....	1:30,000
Streptomycin ..... or	30 μg./ml
Aureomycin	2 μg./ ml.

(Martin, 1950) (Johnson, 1957)

The petriplates will be rotated before the solidification of agar in order to disperse the soil particles evenly. There will be twenty replicates for each data. After pouring and inoculating , the petriplates will be incubated at 28°C and the fungi which will develope after one week, will be examined and identified. The frequency of fungi will be calculated by the following formula:

$$\frac{\text{Number of plates containing a particular fungus}}{\text{Total plates poured.}} \times 100$$

In order to determine the population, the soil held on flattened tip of a needle will be transferred to each plate. Later the average weight of each transfer will be determined. Subsequently the population will be determined by counting the number of colonies developed in all plates and then transferring this

figure to the number of colonies to be developed in one gram of soil.

For determining the relative abundance of the fungi the formula suggested by Mclean and Cook (1957) will be employed:-

$$\frac{\text{Total no. of colonies of a fungus}}{\text{Total no. of colonies of all the fungi}} \times 100$$

For screening nonrhizosphere mycoflora samples will be brought in sterile containers from unsown portion of the beds. Samples will be collected randomly and mixed thoroughly in order to get a composite sample. From this composite sample inoculation will be made by transferring soil with the flattened end of a needle to a sterilized petridishes containing 10 ml of sterilized melted and cooled peptone dextrose agarmedium. Twenty plates will be poured for each data. Before the agar's solidification plates will be rotated in order to make even distribution. The petriplates will then be incubated at 28°C and the fungi which will develop after one week will be examined and identified. R:S ratio will be calculated on the basis of:

$$\frac{\text{Number of Organisms per gram of rhizosphere soil}}{\text{Number of organisms per gram of non-rhizosphere soil}}$$

For screening rhizosplane mycoflora the serial root washing technique of Harley & Waid (1955) , will be adopted . The roots of which rhizosplane mycoflora is to be screened, will be cut into small pieces. These root pieces will be subjected to

30 or more washings in sterile distilled water. The root pieces will then be transferred to sterilized petriplates containing 10 ml melted and cooled peptone dextrose agar medium. The petriplates will be incubated at 28°C for one week. After that the rhizosphere fungi will be identified.

Rhizosphere & Rhizoplane mycoflora of different varieties of Barley & Sorghum :-

Barley seeds of the varieties Jatna, Jyoti, DL-3, RD-31, DL-70, DL-88, RS-6 have been obtained from Indian Agricultural Research Institute , New Delhi. In the case of Sorghum , seeds of varieties CSH-5, Dhanora Local, IS-4776, Nilwa, R-10, Pusachan-1, Rudrapur Local, Vidhisha-60-1, CSV-3, CSV-2, CSH-6, CSV-4 have been obtained from Live Stock Research Centre of G.B.Pant Agriculture University, Pantnagar, Nainital. Studies of rhizosphere and rhizoplane mycoflora of these different varieties of Barley and Sorghum will be screened at maturity . The methods for determining the frequency and population will be same as discussed earlier.

Rhizosphere & Rhizoplane mycoflora of Barley and Sorghum in relation to soil amendments -

The ploughed fields will be treated with different inorganic fertilisers, pesticides and organic manures. Three replicates will be taken for each treatment. The seeds will be sown after one week of the treatment. The dose will be as follows:

Nitrogen	-	100 lb. per acre
Potassium & Phosphate	-	50 lb. per acre
DD	-	40 gallon per acre
Nemagon	-	2 gallon per acre
Thimet	-	10 Kg. per acre
Dasemit	-	10 Kg. per acre
5% Rosgor G	-	10 Kg. per acre

The inorganic fertilizers will be urea, super-phosphate and Murate of potash. The pesticides that will be used are Thimet, Nemagon, Dasemit and 5% Rosgor G. Nemagon will be mixed with appropriate quantity of water and applied with the help of hand injector. The same procedure will be followed for D.D which will be applied directly without emulsifying in water.

The organic amendments will be compost, Bone-meals, Neem cake and ground nut cake.

There will be three replicates (3 beds) for each treatment.

Rhizosphere and rhizoplane mycoflora of Barley and Sorghum in six different soil types :

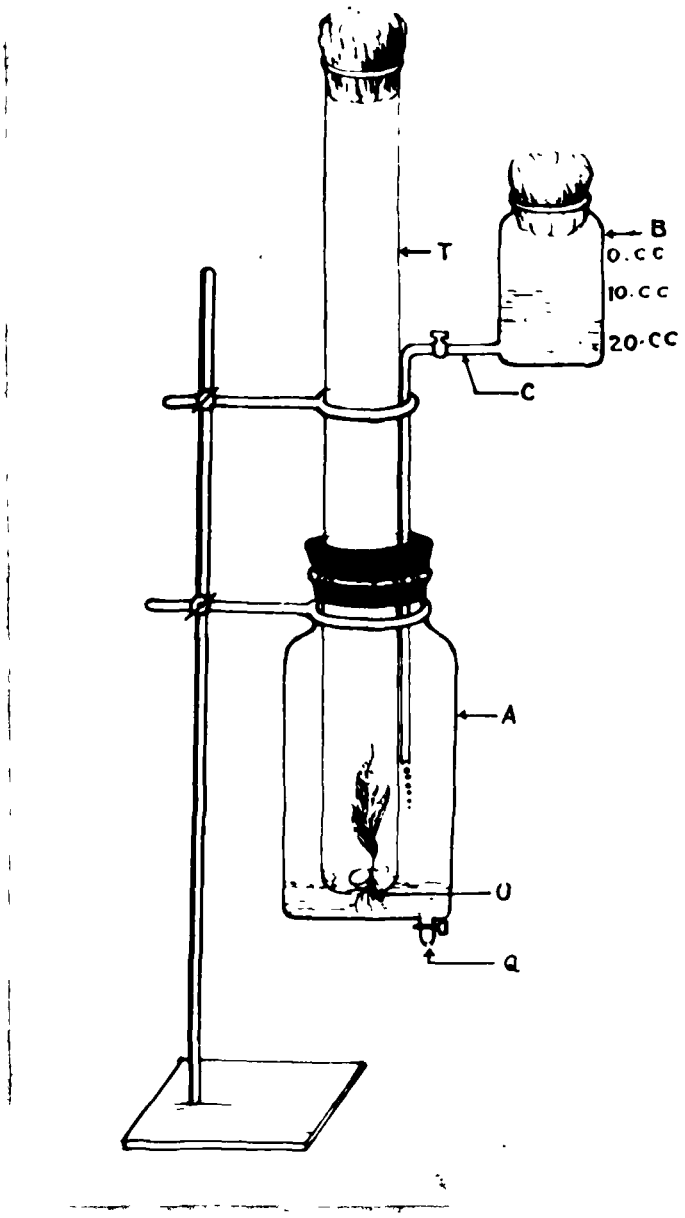
Screening of rhizosphere and rhizoplane mycoflora of Barley and Sorghum will be conducted from six different soil types of Aligarh. These soil types include Eastern upland, Yamuna Khadar, Western Upland, Central low land, Gan ga Khadar and Trans Yam-una Khadar . In order to make rhizosphere and rhsoplane mycoflora analysis plants of Barley and Sorghum

will be collected from these soil types. The technique for screening mycoflora will be the same as discussed earlier.

Effect of foliar spray on rhizosphere and rhizoplane mycoflora of Barley and Sorghum :-

For forliar spray of different concentrations viz., 50, 100 & 150 ppm each of Gibbrelic acid and Maleic hydrazide and Urea will be prepared in sterilised distilled water. Equal lengths of 15 days old plants of Barley and Sorghum will be selected for spraying. Twelve plants will be taken for foliar spray of each concentration of the test chemicals. The chemicals to be sprayed will be applied by a home spray atomiser. At the time of spraying care has to be taken that the solution is sprayed only on the foliage of plants and not on the soil. This can be achieved by covering the soil around the plants by plastic sheets. For dontrol plants will be sprayed with distilled water only. Three spraying will be done at fortnight intervals. Rhizosphere and rhizoplane mycoflora will be screened by the methods discussed earlier.

Apparatus for root exudates



A : Bottle

B : Small bottle

C : Capillary tube

D : Test tube

O : Basal Narrow opening in the test tube

Q : Basal opening in the bottle 'A'

Effect of root exudates obtained from the seedlings of Barley and Sorghum on the germination of spore of certain rhizosphere fungi :

Collection of root exudates:-

Root exudates will be collected with / the help of appratus (D.D Kulshreshtha , 1969) which is shown in the figure.

To a small bottle 'A' fitted with a rubber cork, a Tube 'T' 20 cm long and2.5 cm wide having a narrow basal opening 'O' has been introduced in such a manner that it touches the surface of water in the bottle 'A'. Glass wool will be inserted at the basal end of this tube. Another tube 'C' has also been introduced into the bottle 'A' through a rubber cork and this tube has been connected to another bottle 'B' containing measured amount of water.

After plugging the tube 'T' and bottle 'B' with cotton the whole assembly will be sterilised in an autoclave.

The sterilized seeds of Barley and Sorghum will be introduced under aseptic conditions and will be allowed to germinate. When the roots will emerge and proliferate in water, the deserved amount of root exudate will be removed through the basal opening 'O' of the bottle 'A' in a sterilized flask and stored in a refrigerator. A known quantity of water will be poured again in the bottle 'A' from the bottle 'B' through

the tube 'C' for obtaining further exudates when required.

Collection of spore sphere exudates :

Seeds will be treated with 0.1% mercuric chloride and then washed several times with sterilised water. These seeds will be placed in sterilised pertridishes containing 5 ml of sterilised water. On swelling the seeds will be removed and the remaining water will be spore sphere exudates .

Preparation of Spore suspensions:-

Standard spore suspension of different rhizosphere fungi of concentration 10^5 ml from 7 days old cultures will be prepared for germination test.

Effect of root exudates on the germination of spores:-

About 0.2 ml of Barley and Sorghum root exudates will be obtained from 3, 6 and 10 days old seedlings after growing in the above mentioned apparatus at 20° , 25° , 30°C and then transferred to cavity slides with equal amount of water containing spore suspension. The slides will then be transferred to incubation chamber (Riker and Riker, 1936), and examined under the microscope for germination after 24 hours. Spores contained in 0.4 ml of distilled water will serve as control. There will be three replicates for each treatment.

Effect of root exudates on fungal growth :-

The effect of root exudates on the radial growth of different

fungi will be measured by growing the fungus in 10 ml of PDA to which 5 ml of root exudates of Barley and Sorghum will be added separately. The plates will be inoculated with spore suspension when the mixture will be in solid condition. They will then be incubated at 20°C for 12 days and the growth will be measured in mm after 4,8 and 12 days.

Determination of Aminoacids, sugars and organic acids :-

Root exudates of Barley and Sorghum from six days old seedlings will be collected and examined for the presence of different aminoacids , sugar and organic acids by circular as well as two dimensional chromatographic technique (Ranjan et al , 1955 ; Consden et al ,1944) using n- butanol/ acetic acid / water : : 4 : 1 : 5 for amino acids and sugars; n- butanol/ formic acid / water : : 10 : 2 : 5 for organic acid. The developed chromatograms will be sprayed with 0.2% ninhydrin for aminoacids , anelin hydrogen phosphate for sugars and 0.4% bromophenol blue in 90% alchol for organic acids.

Rhizosphere and rhizoplane mycoflora of healthy and infected plants of Barley and Sorghum :-

Rhizosphere and rhizoplane mycoflora of Barley infected with rust and smut will be studied and compared with healthy plants. The rhizosphere and rhizoplane mycoflora

of Sorghum infected with smut will be analysed and compared with healthy plants. The methods for screening the rhizosphere and rhizoplane mycoflora will be the same as discussed earlier.

Pure cultures of fungi encountered during the investigations will be prepared either by single spore isolation or by hyphal tip isolation. The pure cultures will be finally maintained on agar medium described earlier and will be stored in regrigerator. Fungi will be identified by consulting Thom and Raper (1945), Barnett (1945), Raper and Thom (1949), Clement and Shear (1949) , Gilman (1959).

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REFERENCES

* Absalyamova, R.1963 Effect on plant growth of the complex of root micro-organisms occuring as a result of application of organo-mineral mixtures. Agrobiologiva No.1, 77-81

* Agnihothrudu, V. 1955 State in which fungi occur in the rhizosphere. Nature wissenschaften. 42 : 515 - 516

* Agnihothrudu, V. 1957 Aspergilli from soils of South India Ibid. 44 : 65.

* Agnihothrudu 1953 Soil conditions and root diseases VIII. Rhizosphere microflora of some of the important crop plants of South India. Proc. Indian Acad. Sci., 37 B: 1- 13.

* Babushkina, I.N.1973 Soil microscopic fungi of the rhizosphere of healthy and wilt infected cotton- Gossypium hirsutum L. (Variety 108- F). Fitopatologiya 7 (6) : 525 - 531

Bahadur, P. & S.Sinha 1965 Rhizosphere mycoflora of cluster beans (Cyamopsis psoralioides DC.) in relation to root exudes. Proc. Nat. Acad. Sci., 35 : 343 - 350

Barnett, H.L 1962 Illustrated genera of imperfect fungi (II Edition). Burgess Publishing Co., Minn.USA.

Barton, M. 1957 Germination of Oospore of Pythium mamillatum in response to exudate from living seedlings. Nature, London. 180 : 613- 614

*Berezova, F.F. 1941 Microflora of the rhizosphere of Flax. Microbiology , U.S.S.R. 10 : 918

Bhuvaneswari, K. & M.S.Subba Rao . 1957. Root exudates in relation to the rhizosphere effect. Proc.Indian. Acad. Sci. 45 B : 299-301.

Brian , P.W. 1957: Effect of antibiotics on plants. A. Rev. Pl. Physiol. 8 : 413 - 426 .

Clark, F.E. 1940. Notes on types of bacteria associated with plant roots. Trans. Kansas Acad. Sci. 43 : 75 - 84.

Clark, F.E. 1948. Rhizosphere microflora as affected by soil moisture changes. Soil. Sci. Soc. Am. Proc. 12 : 239 - 242.

Clements, E.S. and C.L Shear (1954). The genera of fungi Hafner Publishing Co., New-York.

Coley-Smith, J.R and C.J Hickman, 1957. Stimulation of Sclerotium germination in Sclerotium cepivorum Berk. Nature , London. 180 : 445

Consden, R., A.H.Gordon and A.J.P.Martin (1944). Qualitative analysis of protein , a paper partition chromatographic method using paper. Biochem. Jour.38: 224

* Craner, M.H.1922. Zur Biochemie und Physiologie der grens schichten lebender Pflanzenzeller. Mel-dinger Norg. Landbrukhoiskole 2 : 1- 160.

Davey, C.B and G.C Papavizas. 1960. Effect of decomposing organic soil amendments and nitrogen on fungi in soil and bean rhizosphere. Trans. Intern. Congr. Soil. Sci. 7th. Congr. (Madison, Wise) Comm, III.p. 551-557.

Dwivedi and Singh, 1971. Fungi in the root region of soybean (Glycin Soia Linn.)I. Effect of foliar spray of Gibberellic acid, maleic hydrazide and urea on the rhizosphere mycoflora. Tropical ecology. 12 (2) : 258 - 263.

Gilman, J.C. 1959 A manmual of soil fungi (Revised IInd. Edition), Oxford & IBH Publishing Co., Calcutta, Bombay, New-Delhi.)

Gujrati, S. 1969. Effect of plant age on rhizosphere micro fungi. Proc. Natn. Acad. Sci. India Sect. B. 38 (2) : 90 - 112.

Harley, T.L. and J.S.Waid, 1955. A method of studying active mycelia on living roots and other surfaces in the soil. Trans. Brit. Mycol. Soc. 38 : 104- 118

Harris, J.R. 1953. Influence of rhizosphere micro organisms on the virulence of Rhizobium trifolii. Nature, Lond. 172 : 507 - 508.

Henderson, V.E. and H.Katznelson. 1961. The effect of plant roots on the nematode population of the soil. Can. J. Microbiol. 7 : 163 - 167

*Hiltner, L. 1904. Über neuere Erfahrungen and probleme auf dem Gebiet der Bodenbak terio logie under besonderer Berucksaich tigung der Grundun gung und Brache. Arb. Dösch. Landev. Ges. 98 : 59 - 78.

Hulse, J.H. and D. Spurgeon. 1974. Triticale. Scientific American. 231 : 72.

* Ishizawa, S., F.Suzuki, O. Sato and H. Toyoda. 1957. Studies on microbial population in the rhizosphere of higher plants with special reference to the method of study. Soil plant Food. 3 : 85 - 94.

Ivarson, K.C. and H. Katznelson. 1960. Studies on the rhizosphere microflora of yellow birch seedlings. Plant. Soil. 12 : 30 - 40.

Johnson, L.F. 1957. Effect of antibiotics on the numbers of bacteria and fungi isolated from soil by dilution plate method. Phytopath. 47 : 630 - 631.

Jordan, V.W.L., B. Sneh, C.B.P.Eddy. 1972. Influence of organic soil amendments on Verticillium dahliae and on the microbial composition of the strawbery rhizosphere. Ann. Appl. Bid. 70 (2) : 139- 148

Katznelson, H. 1946. The rhizosphere effect of mangels on certain groups of soil microorganisms. Soil Sci.62 : 343 - 354.

Katznelson, H., A.G.Lochhead and M.I. Timonin. 1948. Soil microorganisms and the rhizosphere. Bot. Rev. 14 : 543 - 587.

Katznelson, H., J.W.Roult and T.M.B. Payne. 1954. Liberation of aminoacids by plant roots in relation to desiccation. Nature London. 174 : 111.

Khan, A.M. and M.A.Siddiqi. 1962. Fungal population in the soil and on the roots of sugar cane. Indian J.Sug. Res. Dev. 6 : 176 - 178.

Knudson, L. 1920. The secretion of invertase by plant roots. Am.J. Botany. 7 : 371 - 379.

*Krassilnikov, N.A., A.Rybalkina, M. Gabrillian and T. Kondratieva 1933. On the microbiological characteristic of the soils beyond the Volga region. Tr.Komispo Irrigacii Akad. Nauk. U.S.S.R., 5 : 1.

Kulshreshtha, D.D. 1969. Ph.D. Thesis, Aligarh Muslim University Aligarh.

Lochhead, A.G. 1959. Rhizosphere microorganisms in relation to root disease fungi. p. 327- 338. In C.S.Holton et al.(ed.) Plant Pathology, problems and progress 1908-1958. University of Wisconsin Press, Madison, Wisc.

*Louw, H.A., and D.M.Webley, 1959. The bacteriology of the root region of the Oat plant grown under controlled pot culture conditions. J.appl. Bacteriol. 22 : 216 - 226.

*Lyon, T.L. and J.K.Wilson. 1921. Liberation of organic matter by roots of growing plants. New York Agr. Expt.Sta (Geneva, N.Y) Mem. 40 : 1 - 44.

* Maliszewska, W., and R.Moreau. 1959. The rhizosphere of white spruce. Compt. Rend. 249 : 303- 305.

Martin, J.P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil. Sci. 69 : 215 - 233.

Mclean, R.S. and W.R.Ivimey Cook 1957. Practical field ecology.George Allen & Unwin Ltd., London.

Miller, R.D. and C.W.Boothroyd. 1962. Seasonal population of rhizosphere fungi associated with corn roots.Phytopath. 52 : 744.

Mishra, R.R. 1972. Effect of certain chemical fertilisers on the rhizosphere mycoflora of Oryza sativa Linn. III. Phosphate fertiliser- Superphosphate. Mycopath Mycol. Appl. 46 (1) : 97- 102.

*Mosolov, I.V., E.K.H. Rempe and V.A.Alexandrovskaya. 1959. The interactions of the higher plant and microorganisms. Agrobiology (U.S.S.R) 3 : 425 - 430.

*Nilsson, P.E., 1957. Lantbruks- Hogskol. Ann. 23 : 175-218.

*Obraztsova, A.A. 1935. The rhizosphere micro-organisms of Batum and soils. Doklady Akad. Nauk. U.S.S.R. 94 : 70 - 71.

*O' Brien, D.G. and E.G Prentice. 1930. An eelworm disease of potatoes caused by Heterodera schachtii Schot. J.Agr. 13 : 415 - 432.

Padma, R. and K.G.Mukherjee 1972. Fungi in the root region of Rauvolfia serpentina and Rauvolfia canescens. Indian phytopath. 25 (2) : 104 - 107.

Panwar , K.S., Saroj Panwar and K.S.Bilgrami.1967. Rhizosphere mycoflora in the sand-dunes of Western Rajasthan Proc. Natl. Acad. Sci. India B.

Papavizas, G.C. and C.B.Davey. 1961. Extent and nature of the rhizosphere of Lupinus. Plant soil 14 : 215 - 236.

Parkinson, D. and J.H. Clarke. 1961. Fungi associated with the seedling roots of Allium porum L. Plant soil. 13 : 384 - 390.

Patel, J.J. and V.N.Iyer. 1961. Micro-organisms associated with the rhizosphere of the cotton plant. Proc.Indian. Acad. Sci. 44 : 1 - 10.

Peterson, E.A. 1958. Observations on fungi associated with plant roots. Can. J. Microbiol. 4 : 257 - 265.

Peterson, E.A. 1958.Seed-borne fungi in relation to colonisation of roots. Can. J. Microbiol. 5 : 579 - 582.

Peterson, E.A. 1961. Observations on the influence of plant illumination on the fungal flora of roots. Can. J. Microbiol. 7 : 1- 6.

Prakash Dhirendra, M.Mahakooralam, Shabbir Ashraf & Abrar M.Khan 1976. Studies on rhizosphere mycoflora of Egg.plant in relation to soil amendments. Acta Botanica Indica 4 : 68 - 70.

Rai.B & V.K. Tewari 1975. Effect of toxic chemicals on soil mycoflora. Acta. Botarica. Indica. 3 (1) : 19- 22.

Ramchandra-Reddy, T.K. 1959. Rhizosphere microflora of pteridophytes. Curr. Sci. 28 : 113 - 114.

Ramchandra-Reddy.T.K. 1959. Foliar spray of urea and rhizosphere microflora of rice (Oryza sativa L.) Phytopath. Z.36 : 286 - 289.

Rangaswami, G. and V.N.Vasantharajan. 1962a. Studies on the rhizosphere microflora of citrus trees. I. Quantitative incidence of microorganisms in relation to root and shoot growth. Can. J. Microbiol. 8 : 473 - 477.

Rangaswami, G. and V.N.Vasantharajan. 1962b. Studies on the rhizosphere microflora of citrus trees. II. Qualitative distribution of the bacterial flora. Can .J Microbiol.8 : 479 - 484.

Rangaswami, G.and V.M.Vasantharajan, 1962c. Studies on the rhizosphere microflora of citrus trees III. Fungal and actinomycete flora of the rhizosphere Can. J Microbiol. 8 : 485 - 489.

Ranjan, Shri. Govindjee and M.M.Laloraya 1955. Chromatographic studies on the amênoacids metabolism of healthy and diseased leaves of Croton sparsiflorum. Proc. Natl. Inst. Sci. India. 21 : 42 - 47.

* Raper , K.B. and C.Thom. 1949. A manmual of Penicillia. Waverly Press Inc. for the Williams and Selkins Co. Baltimore, MD. U.S.A.

Riker, A.J. and A.S Riker. 1936. Introduction to research on plant diseases. Planographed by Johns. Swiftoo Inc. St. Louis, Chicago, New York. Indianapolis.

Roberts, J.L. and E.Roberts. 1939. Soil Sci. 48 : 135- 139.

* Rouatt, J.W. and H.Katznelson. 1961. A study of the bacteria on the root surface and in the rhizosphere soil of crop plants. J.appl. bacterial. 24 : 164 - 171.

Rouatt, J.W., E.A.Peterson, H. Katznelson and V.E.Henderson. 1963. Microorganisms in the root zone in relation to temperature. Can. J. Microbiol. 9 : 227 - 236.

Rouatt. J.W. and H.Katznelson. 1960. Influence of light on bacterial flora of roots. Nature 186 : 659 - 660.

Rovira, A.D. 1956. Plant root excretions in relation to the rhizosphere effect. II. A study of the properties of root exudate and its effect on the growth of micro-organisms isolated from the rhizosphere and control soil Pl. Soil 7 : 195 - 208.

Rovira, A.D. 1959. Root excretions in relation to the rhizosphere effect IV. Influence of plant species, age of plant, light, temperature and calcium nutrition on exudation. Plant soil 11 : 53 - 54.

Rovira, A.D. 1959. Root excretions in relation to the rhizosphere effect IV. Influence of plant species , age of plant, light, temperature and calcium nutrition on exudation. Plant and soil 11 : 53 - 64.

Roy, R.Y and R.S.Dwivedi, 1967. Rhizosphere studies of cultivated legumes. Proc. Indian Sci. Congr.p.294.

* Santsevich, S.A and V.N. Borisova, 1961. Effect of fertilizers on the root microflora of winter wheat. Microbiology (U.S.S.R) 30: 1033 - 1041

Sondhi , J.K. 1964. Rhizosphere studies of gram in relation to Fusarium wilt. Ph.D.thesis , Agra University, Agra.

Srivastava, V.B, R.R.Mishra.1971. Rhizosphere fungal variation in susceptible and healthy wheat. Indian Phytopath. 24 : 784 - 786.

Starkey, R.L. 1929. Some influence of the development of higher plants upon the micro-organisms in the soil. I. Historical and introductory soil Sci. 27 : 319 - 334.

Starkey , R.L. 1929. Some influence of the development of higher plants upon the micro-organisms in the soil I. Historical and introductory. Soil Sci. 27 : 319 - 334.

Starkey. R.L. 1929. Some influence of the development of the higher plants upon the micro-organisms in soil II. Influence of the stage of plant growth upon abundance of organism. Soil Sci. 27 : 355 - 379.

Starkey , R.L. 1929. Some influences of the development of higher plants upon micro-organisms in the soil III. Influence of the stage of plant growth upon some activities of the organisms. Soil Sci. 27 : 433 - 444.

Starkey, R.L. 1951. Some influences of the development of higher plants upon the micro-organisms in the soil II. Influence of proximity to roots on abundance and activity of microorganisms. Soil Sci. 32 : 367 - 393.

Starkey , R.L. 1951. Some influences of the development of higher plants upon the micro-organisms in the soil. Effects of plants upon distribution of nitrates. Soil Sci. 32 : 395 - 404.

* Strzellayk, E. 1961a. Studies on the incidence of certain 'nutritional' and physiological groups of bacteria in rhizosphere and non-rhizosphere soil. Acta microbiol. Polon. 10 : 169 - 180.

Thom, C. and K.B. Raper 1945. A manual of Aspergilli, Baltimore. The Williams and Wilkins Co.

Timonin, M.I. 1940. The interaction of higher plants and soil microorganisms. I. Microbiol population of rhizosphere of seedlings of certain cultivated plants. Can. J. Res. 18 : 307 - 318.

Vaidehi, B.K. 1973. Effect of foliar application of urea on the behaviour of Helminthosporium hawaiense in the rhizosphere of rice. Indian J. of Mycol. & Plant path. 3 (1) : 81- 85.

Venkatesan, R. 1962. Studies on the actinomycete population of paddy soil. Ph.D.thesis, Department Agr. Annamalai University Annamalainagar, South India.

* Voroshilova, E.A. 1956. The effect of the apple tree on the number of soil microorganisms. Microbiology (U.S.S.R) 25 : 670-699.

* Welte, E.and G. Trolldenier. 1961. The influence of hydrogen ion concentration of the soil on the rhizosphere effect Naturwissenschaften 48 : 509.

Zagallo, A.C. and W.B. Bolton. 1962. Studies on the rhizosphere of tall fescue. Ecology 43 : 54- 62.

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* Original not seen.